

Appl. No. 09/914,705
Amdt. dated December 8, 2005
Reply to Office Action of September 8, 2005

PATENT

Amendments to the Specification:

Please replace paragraph beginning on page 20, line 28 with the following amended paragraph:

Prior to the assay for the anti-influenza virus IgA antibody, each well of the 96-well EIA plate was first coated with 100 μ L of HA vaccine (5 μ g/ml) suspended in a coating buffered (10 mM sodium carbonate buffer pH 9.6). After standing at room temperature for 2 hours, the plate was washed with phosphate buffered saline (PBS) - 0.05% ~~Tween-20~~ TWEEN-20 (polysorbate 20). Subsequently, each well was coated with 300 μ L of a blocking solution (PBS containing 1% bovine serum albumin (BSA) and 0.1% NaN₃) to avoid non-specific reactions. After standing at 4 °C overnight, the plate was washed with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20). A 100 μ L aliquot of test sample diluted with the blocking solution was added to each well. An unabsorbed fraction of serum on the column of Protein G Sepharose was used as a sample for the quantification of the anti-influenza virus IgA antibody. After standing at room temperature for 2 hours, the plate was washed with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20). Subsequently, a 100 μ L aliquot of alkaline phosphatase-conjugated goat anti-mouse IgA α -chain antibody (Zymed Laboratories) diluted with a blocking solution was added to each well. After standing at room temperature overnight, the plate was washed with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20). Finally, *p*-nitrophenol phosphate (1 mg/mL; Wako Pure Chemical Industries) dissolved in 10% diethanolamine (pH 9.8) was added to each well to perform color development. After standing at 37 °C for 20 to 30 minutes, the developed color (O.D. at 405 nm) was assayed in a micro-plate reader.

Please replace paragraph beginning on page 22, line 22 with the following amended paragraph:

Subsequently, detection was carried out for the presence of antibodies (IgG and IgA) specific to the adjuvant and IgE. A linked complex between the hydroxy unsaturated fatty acid and bovine serum albumin as a carrier protein was prepared. Each well of the 96-well EIA plate was first coated with a 100 μ L aliquot of solution containing the complex (1 μ g/ml).

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Subsequently, each well of the plate was coated with 300 µl of a blocking solution (PBS containing 5% skimmed milk) for 1 hour to avoid non-specific reactions. Then, 100 µl samples (nasal irrigation liquid) diluted to various concentrations were added to the respective wells for antigen-antibody reaction and the reaction was continued for 1 hour. The plate was then washed 3 times with PBS-0.05% ~~Tween-20~~ TWEEN-20 (polysorbate-20), and then 100 µl of peroxidase-conjugated anti-mouse IgG, IgA, or IgE antibody (1:1000) as a secondary antibody was added thereto and the reaction was continued for one hour. After the plate was washed 3 times with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20), 100 µl of a substrate solution (0.1 M citrate buffer (pH 4) containing 0.003% hydrogen peroxide and ABTS of 0.3 mg/ml) was added thereto. The plate was incubated for 15 minutes for color development. The color (O.D. at 405 nm) was assayed in a micro-plate reader. The result showed that no differences in the absorbance of nasal irrigation liquid were recognized between the group of mice to which the hydroxy unsaturated fatty acid had been administered intranasally and groups of control mice without administration. According to this result, neither antibody (IgG, IgA) specific to the adjuvant nor IgE was detectable.

Please replace paragraph beginning on page 29, line 8 with the following amended paragraph:

Prior to the assay for anti-influenza virus IgG antibody, each well of the 96-well EIA plate was first coated with 100 µL of anti-mouse IgG monoclonal antibody (mAb) (Pharmingen) diluted with a coating buffer (10 mM sodium carbonate bicarbonate buffer (pH 9.6) containing 10 µg/mL BSA). The plate was allowed to stand at 37 °C for 3 hours, and then the solution in each well was discarded. Subsequently, each well was coated with 300 µL of a blocking solution (PBS containing 1% skimmed milk and 0.1% NaN₃) for 1 hour to avoid non-specific reactions. After allowed to stand at 37 °C for 1 hour, the plate was washed with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20). A 100 µL aliquot of test sample diluted with the blocking solution was added to each well. After allowed to stand at room temperature overnight, the plate was washed with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20). Subsequently, a 100 µL aliquot of biotin-labeled HA vaccine (1 µg/mL) diluted with blocking solution was added to each

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well. After allowed to stand while being shaken at room temperature for an hour, the plate was washed with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20). Subsequently, 100 μ L of streptavidin- β -galactosidase diluted with the blocking solution was added to each well. After allowed to stand while being shaken at room temperature for an hour, the plate was washed with ~~PBS-Tween-20~~ PBS-TWEEN-20 (polysorbate-20). Further, 100 μ L of 0.1 mM 4-methylumbelliferyl- β -galactosidase (Sigma) dissolved in 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% BSA and 0.1% NaN_3 was added to each well and then allowed to stand at 37 °C for 2 hours. Finally, 100 μ L of 0.1 M glycine-NaOH buffer (pH 10.3) was added to each well and the reaction was monitored in a fluorescence plate reader (FLOW LABORATORIES) (Ex. 355 nm, Em. 460 nm).